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Are Class I (Pre-Early) Proteins of Bacteriophage T5 Sufficient to Induce Abortive Infection of ColIb$^+$ *Escherichia coli*?

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When T5 bacteriophage infect a colicin Ib-containing host, a variety of membrane changes and inhibition of macromolecular synthesis occur. This work shows that all these changes also occur when a mutant of T5 that can only inject 8% of its DNA is used. This indicates that all the information necessary for the abortive infection is present on this 8% (first-step-transfer) DNA.

Escherichia coli containing the colicinogenic factor ColIb is a nonpermissive host for bacteriophage T5 and its close relative, BF23. In the presence of the plasmid, the initial stages of infection proceed as usual, but the normal infectious process becomes inhibited before the appearance of progeny phage (19-22).

In a productive infection, the T5 genome is injected in two steps (9-13, 15). The first-step-transfer DNA constitutes only 8% of the whole and codes exclusively for class I (pre-early) proteins. These proteins are synthesized from about 1 min until about 10 min after infection (14, 16), and two of them, the products of genes *A1* and *A2*, are required for entry of the remaining second-step-transfer DNA (12, 14, 18). Second-step-transfer DNA, which carries all class II (early) and class III (late) genes, enters the host at about 4 min after adsorption (12, 13). Class II (early) protein synthesis begins about 2 min later (14), phage DNA replication starts at about 9 min into the infectious process (13), and class III (late) proteins first appear at about 13 min (13, 14).

First-step DNA transfer, class I protein synthesis, and second-step DNA transfer all proceed as usual in ColIb$^+$ hosts (19). At a time 6 to 10 min after infection, however, before or just after class II gene expression begins, transcription and translation cease. Furthermore, no phage DNA replication occurs, although the infecting DNA is not broken down.

Host range (h$^-$) phage mutants, able to grow on colicinogenic hosts, have been isolated (1, 17, 19, 21). The T5h$^-$ phage have a mutation in the class I *A3* gene (1) and fail to make a protein with a molecular weight of 12,000 (22). BF23h$^-$ phage have a mutation in the corresponding *P3* gene (1, 17) and make very low levels of two class I proteins.

Colicinogenic hosts bearing chromosomal mutations (7) or containing mutant ColIb plasmids (reviewed in reference 13) that allow productive infection have also been isolated. Hence, the interaction of at least three proteins (one phage, one plasmid, and one host) is necessary for abortive infection.

Herman and Moyer (6) have proposed that a class II protein is also necessary to induce abortive infection. Their experiments show that, under conditions preventing class II protein synthesis, [^{3}H]uridine incorporation into trichloroacetic acid-insoluble products continues longer than expected in ColIb$^+$ hosts. We have here used an alternate approach, employing mutants defective in second-step DNA transfer, to determine whether any class II proteins are indeed necessary to induce the arrest mechanism.

MATERIALS AND METHODS

Organisms and media. Our organisms are characterized in Table 1. Richard Moyer supplied us with the three isogenic bacterial strains used. RM 42 contains no colicin factor; RM 43 contains wild-type ColIb; RM 39 bears a mutant ColIb plasmid (ColIb h$^-$) that renders the host permissive (R. Moyer, personal communication). Further description is included in the accompanying paper (4). D. J. McCorquodale sent to us T5*am*16d and T5*am*H27, each of which contains an amber mutation in the *A1* gene (5) and can only inject 8% of its DNA.

Wild-type T5 was obtained from Rolf Benzinger. Media, growth of bacteria, stock phage preparation, and phage infection were as described in the accompanying paper (4).

Macromolecular synthesis. RNA synthesis was monitored by measuring incorporation of [^{3}H]uridine into trichloroacetic acid-insoluble material at different times after infection. Bacteria were grown in basic growth medium, with 50 μg of cold uridine per ml added. Bacteria were centrifuged, diluted, infected, and rediluted as outlined previously (4). At time zero,

TABLE 1. *Characterization of bacteria and phage*

E. coli strain	Genotype	Plating efficiency relative to CR63 (*su*$^+$)		
		Wild-type T5	T5*am*16d	T5*am*H27
RM 42	W3110 (thy$^-$ ColI^R)[a]	1	10^{-5}	10^{-5}
RM 43	W3110 (thy$^-$ ColI^R ColIb P9)	10^{-7}	$<10^{-9}$	$<10^{-9}$
RM 39	W3110 (thy$^-$ ColI^R ColIb h$^-$)[b]	1	10^{-5}	10^{-5}

[a] ColI^R indicates resistance to the external action of colicin Ib.

[b] ColIb h$^-$ indicates permissive growth of T5.

[^{3}H]uridine (1 μCi/ml, 5 μCi/μmol) was added; at indicated intervals thereafter, 0.9-ml samples were removed and added to 0.1 ml of cold 50% trichloroacetic acid. The 0.4 ml of this mixture was filtered through Whatman glass fiber filters (GF/F) and washed with 15 ml of cold 5% trichloroacetic acid. The filters were dried in an oven at 60°C for about 1 h and counted in a toluene-based liquid scintillation fluid, using a Beckman LS200 counter. The results represent total (cumulative) counts per minute incorporated into samples of approximately 2.2×10^8 bacteria.

Protein synthesis was measured in the same way, except that cold tyrosine (25 μg/ml) was added to the basic growth medium, and [^{3}H]tyrosine (1 μCi/ml, 7.2 μCi/μmol [final activity]) was added at time zero.

Proline and glutamine uptake. In these experiments, the amount of amino acid taken up by the cell in a 30-, 60-, or 90-s pulse was measured at various times after infection. Bacteria were grown in basic growth medium, spun, concentrated, infected, and rediluted as above. At the indicated times, 2-ml samples were removed and chloramphenicol was added to give a concentration of 100 μg/ml. One minute later, 0.9 ml of this mixture was added to 0.1 ml of the labeled amino acid solution. In the proline assay, 1 μCi of [^{3}H]proline (25 μCi/μmol) was present; in the glutamine experiments, 0.5 μCi of [^{14}C]glutamine (5 μCi/μmol) was present. At indicated intervals after infected bacteria were added to the labeled amino acid, 0.4-ml samples were removed, filtered on Whatman glass fiber filters (GF/F), and washed with 8 ml of M9. A positive control was done with uninfected cells; a negative control was done with cells treated for 15 min with NaN_3 (1%) before addition of chloramphenicol. The results are presented here as percentage of uptake in infected cells relative to that of uninfected controls.

αMG uptake. α-Methylglucoside (αMG) uptake experiments used bacteria grown and infected in basic growth medium, as outlined above. At times indicated, 1 ml was removed and spun in an Eppendorf model 3200 centrifuge for approximately 30 s, resuspended in an equal volume of M9, and spun again. After the second spin, the bacteria were resuspended in basic growth medium, modified by having only 18 μg of glucose per ml. A 0.9-ml sample of this suspension was added to 0.1 ml of [^{14}C]αMG solution (1 μCi/ml, 184 μCi/μmol). At indicated intervals, 0.4-ml samples were removed and filtered over Whatman glass fiber filters (GF/F) and washed with 8 ml of plain M9. The filters were dried and counted. A positive uninfected control was done; a negative control was done with cells treated for 15 min before the assay with 0.07 M NaF and 1% NaN_3. The results are presented as percentage of uptake relative to uninfected controls.

Gel analysis. To see what proteins were labeled with radioactive amino acids after infection, infected cells were pulse-labeled for 5 min with 1 μCi of ^{14}C of an amino acid mixture (NEC-445) per ml of culture. The 5-min pulses were initiated at 1, 6, and 11 min postinfection and terminated by addition of 100 μg of chloramphenicol per ml and chilling. The cells were then centrifuged, washed two times, and resuspended in 1/10 volume of Laemmli electrophoresis buffer (8). The samples were then boiled for 5 min. A 20-μl amount of each sample was loaded onto a 15% acrylamide slab gel and electrophoresed for 14 h at 75 V. The dried gel was autoradiographed by exposing it to Kodak XR1 film.

RESULTS

Macromolecular synthesis in T5 wild-type and T5A1$^-$-infected RM 42, RM 43, and RM 39. The T5A1$^-$ phage mutants are eminently suited for our purposes here. As discussed, these mutants cannot produce any class II or class III proteins, since the A1 gene function is required for second-step DNA transfer. A1$^-$ mutants are also unable to shut off class I gene expression, another function controlled by the *A1* gene (16).

Figure 1 represents cumulative [^{3}H]uridine incorporation into trichloroacetic acid-precipitable material in infected bacteria; Fig. 2 shows the results of the analogous experiments with [^{3}H]tyrosine. Uptake of the labeled precursors and incorporation into macromolecules continued for at least 24 min after RM 42 was infected with either wild-type T5 or an A1$^-$ mutant. In the corresponding infections of RM 43 (ColIb), however, incorporation of both uridine and tyrosine into macromolecules stopped at 6 to 12 min after either mutant or wild-type phage was added. The possibility that this shutoff in RM 43 (ColIb) was due to a property of the colicinogenic host unrelated to abortive infection was ruled out by the fact that incorporation of both substances in infected RM 39 (ColIb h$^-$) continued for the duration of the experiments. From these experiments, it is evident that only class I (pre-early) gene products are necessary to induce the abortive cessation of RNA and protein synthesis.

Proline, glutamine, and αMG uptake in T5 wild-type and T5*am*16d-infected RM 42, RM 43, and RM 39. It was shown in the accom-

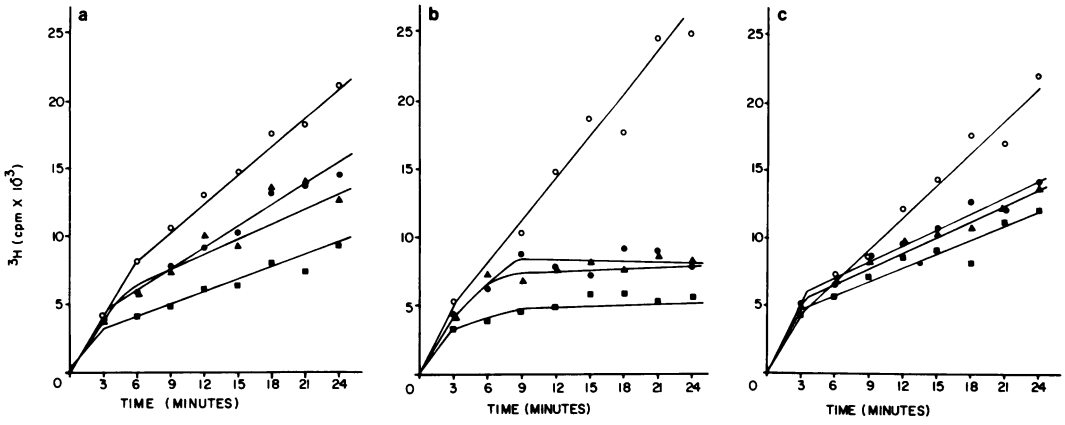

FIG. 1. *RNA synthesis in uninfected and infected E. coli. Cells were grown and infected as detailed in Materials and Methods. [^{3}H]uridine (1 μCi/ml, 5 μCi/μmol) was added at time zero. Samples of 0.9 ml were removed at the indicated times and mixed with 0.1 ml of cold 50% trichloroacetic acid. Acid-insoluble material was collected on glass fiber filters, and the filters were washed. The amount of incorporated radioactivity was determined by liquid scintillation counting. (a) RM 42, which contains no plasmid; (b) RM 43 (ColIb); (c) RM 39 (ColIb h$^-$). Symbols: (○) control, no infection; (■) wild-type T5 infection; (●) T5am16d infection; (▲) T5amH27 infection.*

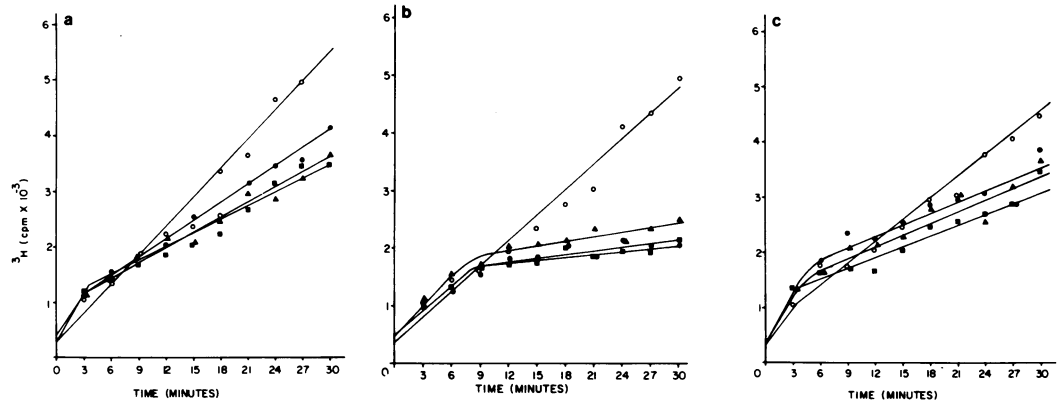

FIG. 2. *Protein synthesis in uninfected and infected E. coli. Cells were grown and infected as described in Materials and Methods. [^{3}H]tyrosine (1 μCi/ml, 7.2 μCi/μmol) was added at time zero. Samples of 0.9 ml were removed at the indicated times and mixed with 0.1 ml of cold 50% trichloroacetic acid. Acid-insoluble material was recovered on glass fiber filters, and the amount of incorporated radioactivity was determined. (a) RM 42; (b) RM 43 (ColIb); (c) RM 39 (ColIb h$^-$). Symbols are as in Fig. 1.*

panying paper (4) that decreased ability of host cells to accumulate proline and glutamine is characteristic of abortive infections, while enhancement of αMG uptake occurs. It is thought that these results can be explained by depolarization of the cell membrane early in the abortive infection. Figures 3, 4, and 5 show the results of experiments comparing the ability of T5*am*16d- and wild-type T5-infected RM 43 (ColIb) and RM 39 (ColIb h$^-$) to transport these three substances. The inhibition of proline and glutamine uptake was the same when both a wild type and an A1$^-$ mutant infected a nonpermissive host. Likewise, the stimulation of αMG transport occurred when both A1$^-$ mutant and wild-type phage were used. Hence, we feel that this conclusively demonstrates that the A1$^-$-infected colicinogenic cells exhibit the same patterns of uptake as do T5 wild-type infections of this host strain and indicates that the membrane changes that occur during a T5 infection of a ColIb$^+$ host also occur when the T5 has injected only 8% of its DNA.

Gel analysis of phage proteins. It was suggested that RM 43 (ColIb) could have an amber suppressor present on the plasmid, invalidating any conclusions regarding infection of these cells with amber mutants. To show that this was not

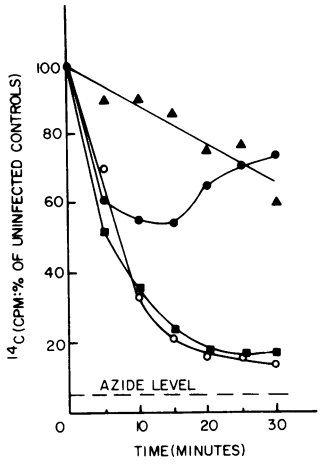

FIG. 3. *Glutamine uptake by infected RM 42, RM 43, and RM 39. Cells were grown and infected as detailed in Materials and Methods. At the indicated times, samples were removed and mixed with [^{14}C]glutamine (0.5 μCi/ml, 5 μCi/μmol). At 30, 60, and 90 s thereafter, samples were removed and filtered through glass fiber filters. The results here are for the 90-s pulses, expressed as a percentage of the amount taken up by uninfected controls in the same period of time. Symbols: (●) T5am16d-infected RM42; (○) T5am16d-infected RM 43 (ColIb); (▲) T5am16d-infected RM39 (ColIb h$^-$); (■) T5 wild-type-infected RM 43 (ColIb).*

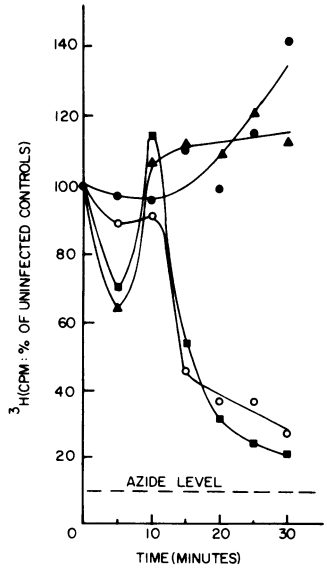

FIG. 4. *Proline uptake by infected RM 42, RM 43, and RM 39. Cells were grown and infected as outlined in Materials and Methods. At the indicated times after infection, samples were removed and mixed with [^{3}H]proline (1 μCi/ml, 25 μCi/μmol). Samples were removed at 30 and 60 s thereafter and filtered through glass fiber filters. The amount of radioactive proline accumulated in 60 s by infected cells is expressed as a percentage of the amount taken up by uninfected controls in the same period of time. Symbols are as in Fig. 3.*

the case, by confirming that class II (early) or class III (late) proteins are not made in A1$^-$-infected ColIb$^+$ cells, we pulse-labeled proteins synthesized from 1 to 6 min, 6 to 11 min, or 11 to 16 min after T5*am*16d infections of the colicinogenic hosts (RM 43). On sodium dodecyl sulfate-polyacrylamide gels, we compared these samples with those of T5*am*16d infections of RM 42 and with T5 wild-type infections of both strains. Only class I (pre-early) proteins were seen during A1$^-$ infections of either host strain (Fig. 6). Further confirmation that T5*am*16d and T5*am*H27 were indeed acting as A1$^-$ mutants ought to act in all strains came from experiments showing that these mutants failed to break down host DNA (data not shown).

DISCUSSION

T5 will not normally grow in *E. coli* containing the ColIb plasmid. There are mutant phage,

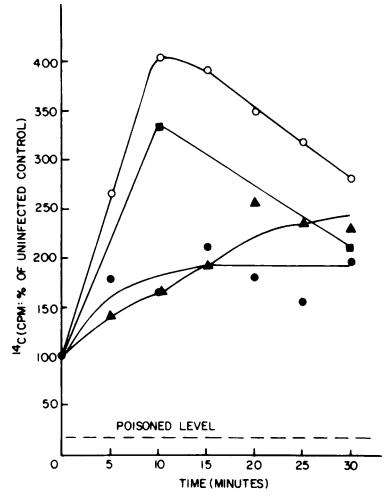

FIG. 5. *αMG uptake by infected RM 42, RM 43, and RM 39. Cells were grown and infected as outlined in Materials and Methods. At the indicated times after infection, samples were removed, spun, and washed with M9. After a second wash, the cells were resuspended in M9 with 18 μg of glucose per ml, and 0.1 ml of [^{14}C]αMG (10 μCi/ml, 184 μCi/μmol) was added to 0.9 ml of the cell suspension. Samples were removed at 45 and 90 s thereafter and were filtered through glass fiber filters. The results here are for the 90-s pulses, expressed as a percentage of the amount taken up by uninfected controls in the same period of time. Symbols are as in Fig. 3.*

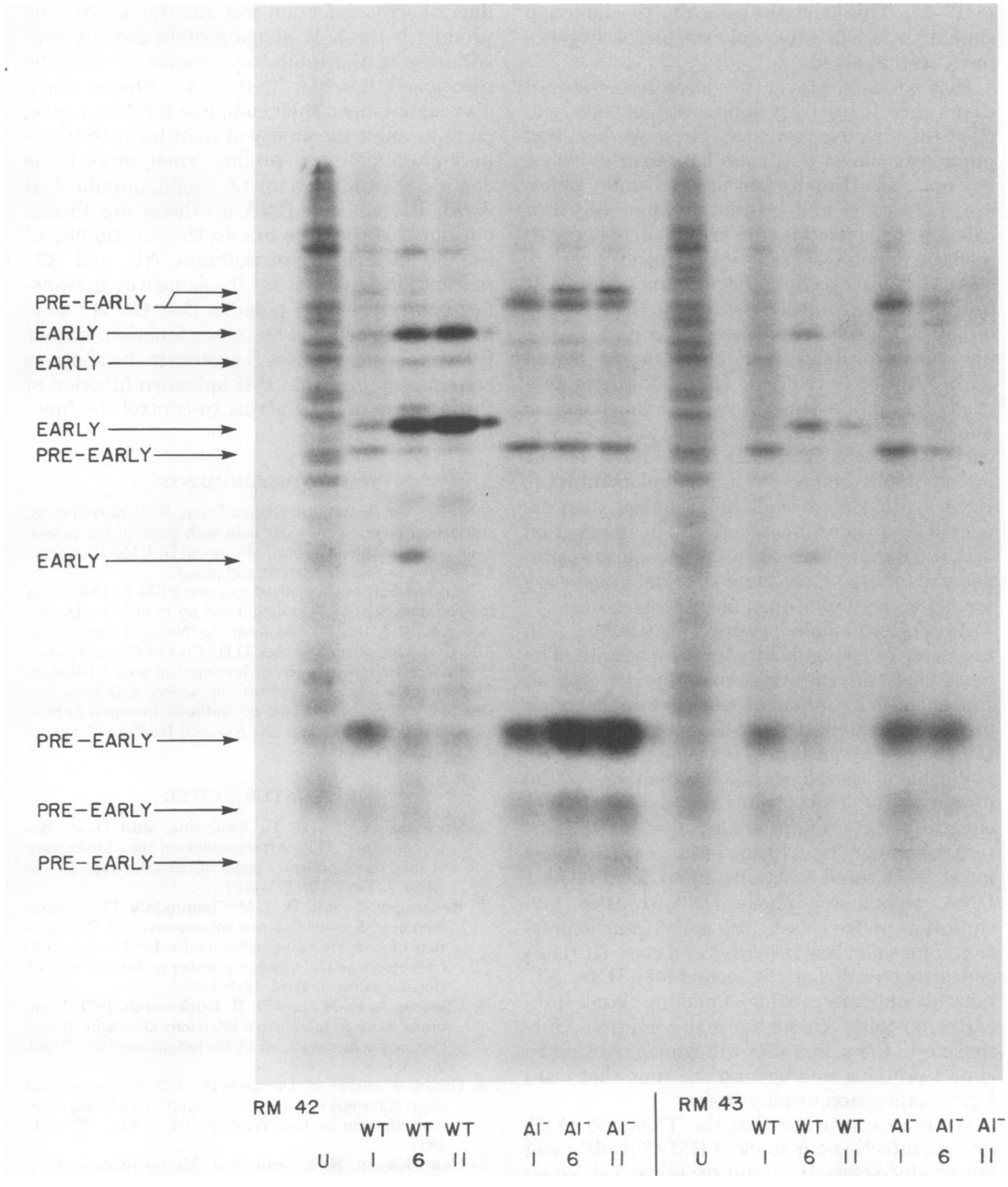

FIG. 6. *Polyacrylamide gel electrophoresis of pulse-labeled proteins at various times after infection. Cells were grown and infected in a synthetic medium. At the times indicated, RM 42 or RM 43 (ColIb) infected with either T5 or T5A1⁻ was labeled with 1 μCi of a ^{14}C-labeled amino acid mixture per ml. Five minutes later, chloramphenicol was added and the cells were chilled. They were then centrifuged, washed, resuspended in 1/10 volume of electrophoresis buffer, and boiled for 5 min. Electrophoresis of 20-μl samples was on a 15% slab gel for 14 h at 75 V. The dried gel was autoradiographed by exposing it to Kodak XR1 film.*

however, that can overcome the block caused by the plasmid and produce progeny in spite of the plasmid's presence. These mutant phage are deficient in a small class I protein (22). Presumably, this protein, the product of the *A3* gene, functions in conjunction with a plasmid-coded protein(s) and a chromosomally coded protein(s) to lead to shutdown of the T5 infectious process

(3, 17–22). This being the case, abortive infection should also occur when only the pre-early genes have been injected.

Herman and Moyer (6) have hypothesized that a class II (early) protein is also necessary to elicit the abortive response. These workers had previously shown that class I (pre-early) genes, but not class II (early) or class III (late) genes, are transcribed and translated when very low calcium concentrations are present in the growth medium. Upon elevation of Ca^{2+} concentration, class II RNA synthesis begins immediately. If ColIb$^+$ hosts are infected with T5 in the presence of low calcium, allowed time to produce class I (pre-early) proteins, and then supplemented with additional calcium, RNA synthesis proceeds for only a short time; i.e., the infection aborts. If the same experiment is performed, except that chloramphenicol is added to the infected cells before calcium supplementation, RNA synthesis proceeds much longer than expected for an abortive infection. The implication is that chloramphenicol, in the second case, prevents synthesis of the class II protein necessary for the activation of the abortive mechanism.

As McCorquodale has previously stated (13), however, other explanations are possible. The most likely alternative is that the low calcium concentration present at the time of infection might also inhibit, to some degree, production of the class I (pre-early) *A3* (*h*) gene product.

We have, therefore, taken advantage of the properties of T5A1$^-$ mutants to see whether class II (early) proteins are involved in the abortive infection. The *A1* gene product is necessary for at least three functions: breakdown of host DNA, second-step phage DNA transfer, and shutoff of phage class I (pre-early) gene expression. Since all class II (early) and class III (late) genes are encoded on the second-step DNA, A1$^-$ mutants only are capable of making class I (pre-early) proteins. Under normal conditions, furthermore, these mutants will continue to make class I proteins well beyond the time that class I gene expression usually stops.

We have confirmed that the T5*am*16d (A1$^-$) mutant infecting our stock of RM 43 (ColIb) will not produce class II (early) proteins. Yet, when this or another A1$^-$ mutant infects colicinogenic hosts, transcription and translation of the class I (pre-early) genes stop at the time characteristic of abortive infections. Furthermore, changes in ability of the infected colicinogenic hosts to accumulate several compounds develop at the same time as in the abortive infections of the wild-type T5.

We believe that our studies demonstrate that no class II (early) proteins are necessary to induce abortive infection and that the *A3* (*h*) gene product is the only phage protein directly contributing to the inhibitory process. It must be mentioned, however, that T5A2$^-$ phage, which also cannot inject their complete DNA molecule, do not inhibit the ability of their hosts to transport glutamine and proline when these hosts contain the ColIb factor (J. Glenn, unpublished data). Protein and RNA synthesis are turned off, but this could be due to the functioning of the *A1* gene in these mutants. A1$^-$ and A2$^-$ mutant DNAs do not act the same way in transfections (2), so it is possible that the *A2* gene product has an as yet unknown function. Rather than implicating a class II protein in the abortive response, we feel that this unknown function of the *A2* gene may be acting to control the function of the *h* gene.

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